

Appl. No. : 10/617,915  
Filed : July 10, 2003

### AMENDMENTS TO THE CLAIMS

1 – 18 (Canceled).

19. (Currently Amended) A method for monitoring the concentration of one or more metabolites or analytes, the method comprising:

applying a skin sensor composition to a surface of the skin for a predetermined period of time, wherein said skin sensor composition comprises one or more of a reporter dye and a marker dye; or a dye exhibiting wavelength shift in absorption or fluorescence emission in the presence of a metabolite;

causing penetration of the skin sensor composition to a depth of about 10  $\mu\text{m}$ , wherein said depth corresponds with the bottom of the dead stratum corneum layer, to about 175  $\mu\text{m}$ , wherein said depth corresponds with the top of the dermal layer, into the epidermis; and

monitoring a change in the intracellular concentration of the one or more metabolites or analytes in a metabolic pathway by detecting changes in one or more reporter dyes at one or more time points using an optical reader.

20. (Canceled).

21. (Canceled).

22. (Canceled).

23. (Original) The method of claim 19, wherein the skin sensor composition comprises a mitochondrial stain sensitive to membrane potential or chemical gradient.

24. (Original) The method of claim 19, wherein the skin sensor composition comprises a dye or stain that transfers energy from a molecule generated as a result of the oxidative metabolic pathway and that has a stoichiometric or highly correlated relationship with glucose concentration.

25. (Original) The method of claim 23, wherein the mitochondrial stain is a polycyclic aromatic hydrocarbon dye selected from the group consisting of: rhodamine 123; di-4-ANEPPS; di-8-ANEPPS; DiBAC4(3); RH421; tetramethylrhodamine ethyl ester, perchlorate; tetramethylrhodamine methyl ester, perchlorate; 2-(4-(dimethylamino)styryl)-N-ethylpyridinium iodide; 3,3'-dihexyloxacarbocyanine, 5,5',6,6'-tetrachloro-1,1',3,3' -tetraethyl-benzimidazolylcarbocyanine chloride; 5,5',6,6'-tetrachloro-1,1',3,3' -tetraethyl-benzimidazolylcarbocyanine iodide; nonylacridine orange; dihydrorhodamine 123

dihydrorhodamine 123, dihydrochloride salt; xanthene; 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein; benzenedicarboxylic acid; 2(or 4)-[10-(dimethylamino)-3-oxo-3-H-benzo[c]xanthene-7-yl]; and iodine dissolved in potassium iodide.

26. (Original) The method of claim 19, wherein the skin sensor composition comprises a dye selected from the group consisting of: coumarin; derivatives of coumarin; anthraquinones; cyanine dyes; azo dyes; xanthene dyes; arylmethine dyes; pyrene derivatives; and ruthenium bipyridyl complexes.

27. (Original) The method of claim 19, wherein the one or more metabolites or analytes is selected from the group consisting of: lactate; hydrogen ion ( $H^+$ ); calcium ion ( $Ca^{2+}$ ) pumping rate; magnesium ion ( $Mg^{2+}$ ) pumping rate; sodium ion ( $Na^+$ ) pumping rate; potassium ion ( $K^+$ ) pumping rate; adenosine triphosphate (ATP); adenosine diphosphate (ADP); the ratio of ATP to ADP; inorganic phosphate ( $P_i$ ); glycogen; pyruvate; nicotinamide adenine dinucleotide phosphate, oxidized form ( $NAD(P)^+$ ); nicotinamide adenine dinucleotide phosphate, reduced form ( $NAD(P)H$ ); flavin adenine dinucleotide, oxidized form (FAD); and flavin adenine dinucleotide, reduced form ( $FADH_2$ ); and oxygen ( $O_2$ ) utilization.

28. (Original) The method of claim 19, wherein the skin sensor composition is formulated as any one or more of the following: an emulsion, an ointment, a disposable gel film patch, a reservoir device, a cream, a paint, polar solvents or non-polar solvents.

29. (Original) The method of claim 19, wherein the penetration of the skin composition is accomplished using an active transport technique or a passive transport technique selected from the group consisting of: electroporation, laser poration, sonic poration, ultrasonic poration, iontophoresis, mechanical-poration, solvent transport, tattooing, wicking, and pressurized delivery.

30. (Original) The method of claim 19, wherein the penetration of the skin sensor composition to a depth of about 10  $\mu m$  to about 175  $\mu m$  is accomplished by combining the composition with molecular size attachments.

31. (Currently amended) The method of claim 19, where the predetermined period of time is selected from the group consisting of at least 24~~[-48]~~ hours, at least 2~~[-6]~~ hours, from about 5 seconds to 5 minutes, and from about 30 seconds to 5 minutes.

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32. (Original) The method of claim 19, where monitoring the change in metabolite or analyte concentration comprises detecting at least one wavelength above 450 nm.

33. (Currently amended) A method for monitoring in vivo blood glucose levels, the method comprising: applying the a skin sensor composition to a surface of the skin for a predetermined period of time, wherein said skin sensor composition comprises one or more of a reporter dye and a marker dye; or a dye exhibiting wavelength shift in absorption or fluorescence emission in the presence of a metabolite;

causing penetration of the skin sensor composition to a depth of about 10  $\mu\text{m}$ , wherein said depth corresponds with the bottom of the dead stratum corneum layer, to about 175  $\mu\text{m}$ , wherein said depth corresponds with the top of the dermal layer, into the epidermis;

monitoring a change in the intracellular concentration of the one or more metabolites or analytes by detecting changes in the reporter dye using an optical reader, and

correlating the change in the intracellular concentration of the one or more metabolites or analytes with in vivo blood glucose levels.

34. (Original) The method of claim 33, wherein the skin sensor composition comprises a mitochondrial vital stain or dye, or a dye exhibiting redox potential or energy transfer properties.

35. (Original) The method of claim 34, wherein the mitochondrial vital stain or dye is at least one polycyclic aromatic hydrocarbon dye selected from the group consisting of: Rhodamine 123, Di-4-ANEPPS; Di-8-ANEPPS, DiBAC4(3), RH421, Tetramethylrhodamine ethyl ester, perchlorate, Tetramethylrhodamine methyl ester, perchlorate, 2-(4-(dimethylamino)styryl)-N-ethylpyridinium iodide, 3,3'-Dihexyloxacarbocyanine, 5,5',6,6'-tetrachloro-1,1',3,3' - tetraethyl-benzimidazolylcarbocyanine chloride, 5,5',6,6'-tetrachloro-1,1',3,3' -tetraethyl-benzimidazolylcarbocyanine iodide, Nonylacridine Orange, Dihydrorhodamine 123 and Dihydrorhodamine 123, dihydrochloride salt; xanthene; 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein; benzenedicarboxylic acid; 2(or 4)-[10-(dimethylamino)-3-oxo-3-H-benzo[c]xanthene-7-yl]; and iodine dissolved in potassium iodide.

36. (Original) The method of claim 33, wherein the skin sensor composition comprises at least one dye selected from the group consisting of: coumarin, derivatives of

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coumarin, anthraquinones, cyanine dyes, azo dyes, xanthene dyes, arylmethine dyes, pyrene derivatives, and ruthenium bipyridyl complexes.

37. (Original) The method of claim 33, wherein the one or more metabolites or analytes is selected from the group consisting of: lactate; hydrogen ion ( $H^+$ ); calcium ion ( $Ca^{2+}$ ) pumping rate; magnesium ion ( $Mg^{2+}$ ) pumping rate; sodium ion ( $Na^+$ ) pumping rate; potassium ion ( $K^+$ ) pumping rate; adenosine triphosphate (ATP); adenosine diphosphate (ADP); the ratio of ATP to ADP; glycogen; pyruvate; nicotinamide adenine dinucleotide phosphate, oxidized form ( $NAD(P)^+$ ); nicotinamide adenine dinucleotide phosphate, reduced form ( $NAD(P)H$ ); flavin adenine dinucleotide, oxidized form (FAD); flavin adenine dinucleotide, reduced form ( $FADH_2$ ); and oxygen ( $O_2$ ) utilization.

38. (Original) The method of claim 33, wherein the skin sensor composition is formulated as an emulsion, cream, ointment, disposable gel film patch, reservoir device, paint, or solvent mixture.

39. (Original) The method of claim 33, wherein the penetration of the skin composition is accomplished using at least one active transport or passive transport technique selected from the group consisting of: electroporation, laser poration, sonic poration, ultrasonic poration, solvent transport, iontophoresis, mechanical-poration, tattooing, painting, wicking and pressurized delivery.

40. (Original) The method of claim 33, wherein the penetration of the skin sensor composition to a depth of about 10  $\mu m$ , wherein said depth corresponds with the bottom of the dead stratum corneum layer, to about 175  $\mu m$ , wherein said depth corresponds with the top of the dermal layer, is accomplished by combining the composition with molecular size attachments.

41. (Currently amended) The method of claim 33, where the predetermined period of time is selected from the group consisting of at least 24~~[-48]~~ hours, at least 2~~[-6]~~ hours, from about 5 seconds to 5 minutes, and from about 30 seconds to 5 minutes.

42. (Original) The method of claim 33, where monitoring the change in the one or more metabolite or analyte concentrations comprises measuring at least one spectral emission at a wavelength above 450 nm.

43. (Original) The method of claim 33, wherein the one or more metabolites are selected from the group consisting of: lactate; hydrogen ion ( $H^+$ ); calcium ion ( $Ca^{2+}$ ) pumping

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rate; magnesium ion ( $Mg^{2+}$ ) pumping rate; sodium ion ( $Na^{+}$ ) pumping rate; potassium ion ( $K^{+}$ ) pumping rate; adenosine triphosphate (ATP); adenosine diphosphate (ADP); the ratio of ATP to ADP; glycogen; pyruvate; nicotinamide adenine dinucleotide phosphate, oxidized form ( $NAD(P)^{+}$ ); nicotinamide adenine dinucleotide phosphate, reduced form ( $NAD(P)H$ ); flavin adenine dinucleotide, oxidized form (FAD); flavin adenine dinucleotide, reduced form ( $FADH_2$ ); and oxygen ( $O_2$ ) utilization.

44- 53 (Canceled).